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Safranin O-Stained Antigen Microagglutination Test for Detection of Brucella Antibodies

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A microagglutination test was compared with a standard tube agglutination test for the detection of brucella antibodies. Advantages of the microagglutination test were that it required less time to perform, had a shorter incubation period, and used less antigen.

The tube agglutination test (TAT) is the most widely used laboratory test for the detection of brucella antibodies in human subjects. Microagglutination tests (MATs) have been described by De Mello and De Mello (2), Elek and Vizy (3), and Gaultney et al. (4). The MAT requires less time to perform and uses less antigen than the TAT. We recently described an MAT for the detection of *Francisella tularensis* antibodies which uses a safranin O-stained antigen (1). The present report describes an MAT for the detection of brucella antibodies.

The human serum specimens used in the TAT and MAT were sent to our laboratory by various state health departments for the determination of brucella antibody titers. Of the specimens studied, 50 were negative (TAT titers <160) and 50 were positive (TAT titers ≥160). The high, low, and negative reference (control) sera were obtained from the Biological Products Division of the Center for Disease Control.

The stock suspension of *Brucella abortus* strain 1119-3 antigen was prepared and standardized by the National Animal Disease Laboratory, U.S. Department of Agriculture, Ames, Iowa. The working dilution of antigen for the TAT was a 1:50 dilution of the stock suspension in phenolized (0.5%) saline. The working dilution of antigen for the MAT was a 1:50 dilution of the stock suspension in phosphate-buffered saline (pH 7.2), containing a final concentration of 0.005% safranin O. The safranin O-phosphate-buffered saline solution was prepared by adding 1 ml of a 0.5% aqueous stock solution of safranin O to 99 ml of phosphate-buffered saline, pH 7.2.

The TAT procedure was that described by Spink et al. (7). Doubling dilutions of serum were made in 0.85% saline in tubes (13 mm by 100 mm), starting with a 1:10 dilution and going to 1:5120. High, low, and negative reference sera of known titers were included as controls. Each tube contained 0.5 ml of diluted serum to which an equal amount (0.5 ml) of the 1:50 dilution of antigen (working dilution) was added. The contents of the tubes were mixed, and the tubes were incubated in a 37°C water bath for 48 h.

The MAT procedure is a modification of the one described by Gaultney et al. (4). Rigid V-bottomed microtitration plates were marked off with one row of 12 wells assigned to each specimen, and eight specimens were assigned to each plate. High, low, and negative reference sera of known titers were included in each day's run, as well as an antigen control. Phosphate-buffered saline, pH 7.2, diluent (no safranin) was added to wells 2 through 12 in each row in 0.05-ml amounts with a calibrated pipette dropper. A 1:10 dilution of each serum specimen was made by adding 0.1 ml of serum to 0.9 ml of phosphate-buffered saline, pH 7.2 (no safranin), in a small test tube. After mixing, 0.1 ml of the 1:10 dilution of serum was added to the first well of each row of the microtitration plate, and doubling dilutions were made with an automatic diluter (Dynatech Corp.). An equal amount (0.05 ml) of the safranin O-stained antigen was added to each well. The serum dilutions were doubled by the addition of antigen so that the dilution in the first well of each specimen was 1:20 instead of 1:10. The plates were sealed with transparent tape plate sealers (Dynatech Corp.) to prevent evaporation. The contents of the plates were mixed either on a vertical vibrator (Arthur H. Thomas Co.) for 20 s or by hand by tapping the edges of the plate for 20 s. After mixing, the plates were incubated for 24 h in a 37°C incubator. They were read on a test-reading mirror (Dynatech Corp.) under a fluorescent lamp. A thin piece of translucent paper or facial tissue was placed over the top of the plate to facilitate reading. The

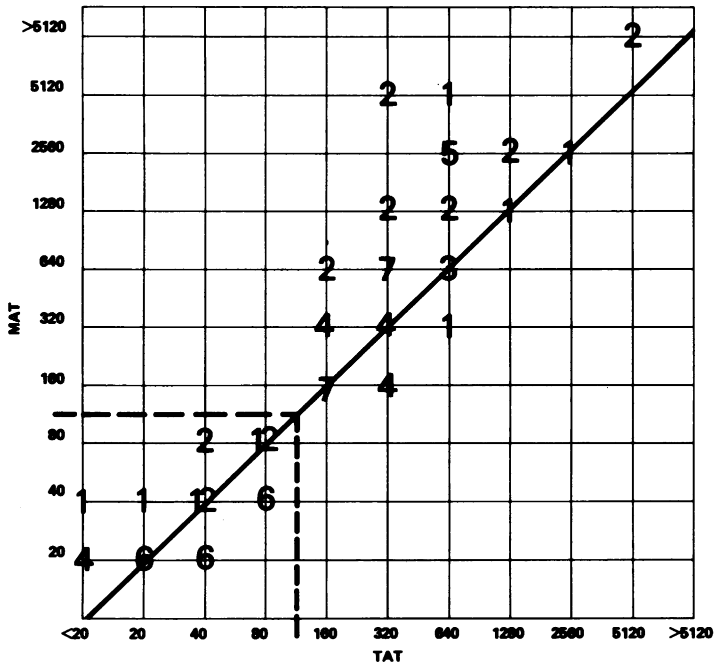

FIG. 1. *Distribution of TAT and MAT titers.*

TABLE 1. *Comparison of TAT and MAT titers of serum specimens*

Titer	TAT[a]	MAT[a]					
		1 dilution lower	Same titer	1 dilution higher	2 dilutions higher	4 dilutions higher	>4 dilutions higher
<20	5			4	1		
20	7		6	1			
40	20	6	12	2			
80	18	6	12				
160	13		7	4	2		
320	19	4	4	7	2	2	
640	12	1	3	2	5		1
1,280	3		1	2	0		
2,560	1		1				
5,120	2						2

[a] Data are the number of specimens with the given titer.

absence of agglutination was indicated by a large button of red-stained cells in the center of the well, surrounded by clear pink diluent. Agglutination was indicated by a mat of stained cells covering the bottom of the well or by a diminished button of cells in the center of the well surrounded by a slightly opaque diluent. The difference could be seen in the reactions of the positive and negative reference sera.

A fourfold increase in titer between acute- and convalescent-stage paired serum specimens with the TAT or MAT was the most significant indication of infection. Single-specimen titers ≥160 with the TAT or MAT are suggestive of infection (positive) at an unknown time (6). Titers <160 were inconclusive with both tests. For purposes of this paper, titers ≥160 were positive and titers <160 were negative.

Table 1 gives a comparison of TAT and MAT titers. The TAT and MAT titers agreed within ±1 dilution step with 49 of the 50 negative specimens (98% agreement). The TAT and MAT titers agreed within ±1 dilution step with 36 of the 50 positive specimens (72% agreement).

There were 14 positive serum specimens (28%) whose MAT and TAT titers did not agree within ±1 dilution step. This disagreement was due entirely to the specimens having MAT titers ≥2 dilutions higher than TAT titers. None of the TAT titers was ≥2 dilutions higher than the MAT titers. Usually a microagglutination technique gives higher titers than a macrotechnique with specimens with elevated antibody levels, whereas the titers are in close agreement for specimens with low antibody levels (1, 4, 5). The geometric mean titer for the positive serum specimens (≥160) was significantly higher for the MAT than for the TAT (777.1 as compared with 399.5); the geometric mean titer for the negative serum specimens was not significantly different for the two tests (38.9 as compared with 40.5).

Figure 1 shows the distribution of titers with the two tests. The difference between the titers obtained with the two tests with negative specimens and with positive specimens is evident. There was little difference between the two tests with the 50 negative specimens; 68% had MAT titers ≥40 and 71% had TAT titers ≥40. However, 60% of the 50 positive specimens had MAT titers ≥640, whereas only 36% had TAT titers ≥640.

The MAT is preferred to the TAT for the detection of brucella antibody titers because it requires less time to perform, has a shorter incubation time, and uses less antigen than the TAT.

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